PCR product clean-up (Spin Column Extraction)

NucleoSpin® PCR clean-up Gel Extraction Kit

1. DNA Purification

a. Adjust DNA binding condition, For very small sample volumes < 30 µL adjust the volume of the reaction mixture to 50–100 µL with water. It is not necessary to remove mineral oil. Mix 1 volume of sample with 2 volumes of Buffer NTI (e.g., mix 100 µL PCR reaction and 200 µL Buffer NTI).
(Note: For removal of small fragments like primer dimers dilutions of Buffer NTI can be used instead of 100 % Buffer NTI. Please refer to section 2.3. Incubate the mixture at 50°C for 5-10 min or until the gel has completely melted.)

b. Place a NucleoSpin® PCR clean-up Gel column in a provided 2 ml collection tube. Apply up to 700 µl of the DNA/agarose solution to the NucleoSpin® PCR clean-up Gel column, and centrifuge at 11, 000 x g for 30s at room temperature.

c. Discard liquid and place the NucleoSpin® PCR clean-up Gel column back into the same collection tube. For volumes greater than 700 µl, load the column and centrifuge successively, 700 µl at a time. Each NucleoSpin® PCR clean-up Gel column has a total capacity of 25 µg DNA. If the expected yield is larger, divide the sample into the appropriate number of columns.

d. Add 700 µl of Buffer NT3 into the NucleoSpin® PCR clean-up Gel columnCentrifuge at 11,000 x g for 30s at room temperature to wash the column.Discard the flow-through and re-use the collection tube.

e. Repeat step k with another 700µl of Buffer NT3

f. Discard liquid and centrifuge the empty NucleoSpin® PCR clean-up Gel column for 1 min at 11,000 x g to dry the column matrix. Do not skip this step, it is critical for the removal of ethanol from the NucleoSpin® PCR clean-up Gel column.

g. Place a NucleoSpin® PCR clean-up Gel column into a clean 1.5 ml eppendorf tube. Add 15-30µl (depending on desired concentration of final product) of Elution Buffer (10 mM Tris-HCl, pH 8.5) directly onto the column matrix and incubate at room temperature for 1 minute. Centrifuge for 1 min at 11,000 x g to elute DNA. This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.